Some Changes in the Endosperm Proteins during Sprouting of Wheat[1]

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ABSTRACT

Effects of sprouting of wheat on endosperm proteins and some related technological properties were investigated for one variety of hard red spring wheat. Quantitative and qualitative changes in the endosperm protein occurred during sprouting. Solubility fractionation showed a marked decrease in the amount of insoluble residue protein. The decrease in the amount of the insoluble protein component and the increase in the number of amino groups were related to the increase in proteolytic activity of the flours. Proteolytic activity of the flour increased 17-fold during 8 days of sprouting. The increase in the number of amino groups was taken as evidence of proteolytic cleavage of peptide bonds. Analyses of SH and S-S groups did not show any significant changes in these groups when wheat was sprouted for 8 days. They do not appear to be involved in the observed degradation of the insoluble endosperm protein.

The detrimental effect of premature sprouting of wheat on baking quality of the flour milled from such wheat has been known for many years. This detrimental effect is generally attributed to high amylase activity, especially α-amylase which develops in the grain during sprouting (1). In addition to the marked increase of α-amylase activity during sprouting, many cereal chemists (2,3,4,5) have observed that there is also a significant increase in proteolytic activity. The possible detrimental effect of this enzyme on breadmaking quality is obvious although generally it is not considered to be as serious as that of α-amylase. Furthermore, it is known that gluten from sprouted wheat is too extensible for optimal baking quality. Work of Beresh (4) and Redman (6) presents evidence that the rapid softening of gluten washed from flour milled from a grist that contained small quantities of sprouted wheat is due to proteolytic hydrolysis of the gluten proteins. An earlier publication of Shorina et al. (7) suggested that the softening of gluten is caused by the reduction of disulfide cross-links by protein disulfide reductase which develops during sprouting. So far as the authors are aware, there have been no reports on the changes of the physical properties of the endosperm proteins of bread wheats during sprouting.

This article reports results of a study of some physicochemical properties of the endosperm proteins that might be related to breadmaking quality of flour milled from sprouted wheat. A companion article (8) dealt with changes in the starch and α-amylase activity in the same wheat samples.

MATERIALS AND METHODS

Experimental Flours

One variety of Canadian hard red spring wheat (Manitou) was permitted to sprout to three different levels under laboratory conditions. The wheat was soaked in three lots of distilled water at 10°C. for 2 days and then germinated and sprouted at 20°C. for various times. After 0, 2, 4, and 8 days, the wheats were

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freeze-dried to about 5% moisture and milled into straight-grade flour on a Buhler experimental mill after tempering overnight to 15.5% moisture. Some analytical and technological data for the five flours used in this study are given in Table I.

Fractionation of Flour Proteins

Flour proteins were fractionated into solubility fractions by the modified Osborne technique described by Chen and Bushuk (9). This fractionation gives five protein groups: 1) water-soluble, 2) salt-soluble, 3) 70% ethanol-soluble, 4) acetic acid-soluble, and 5) insoluble residue. Total extracts and solubility fractions were also fractionated by column chromatography on Sephadex G-150, using acetic acid-urea-cetyltrimethyl ammonium bromide solution (AUC) as eluting solvent, into four groups according to molecular weight, using the procedure of Meredith and Wren (10). The four fractions obtained by the chromatography procedure will be identified by Roman numerals I to IV in order of decreasing molecular weight.

Polyacrylamide Gel Electrophoresis

Three of the five solubility fractions and all four fractions obtained by chromatographic fractionation of total AUC extracts were examined by polyacrylamide gel electrophoresis (PAGE) using the procedure of Chen and Bushuk (11).

Proteolytic Activity

Proteolytic activities of two different extracts of the flours and the five fractions obtained by solubility fractionation were determined using the Chua (12) modification of the Ayre-Anderson method (13). One extract was obtained using a solution of ammonium sulfate (10% saturation) and the other using 0.1M acetate buffer of pH 3.8.

Analytical Procedures

Amino acid compositions were determined on a Beckman model 121 automatic

TABLE I. ANALYTICAL AND TECHNOLOGICAL DATA ON EXPERIMENTAL FLOURS

	Control	Soaked	Germinated		
			2 Days	4 Days	8 Days
Yield, %	73.5	75.1	71.9	71.9	63.8
Ash, % (14% m.b.)	0.40	0.36	0.25	0.29	0.27
Color, units	0.6	0.1	0.6	0.1	2.7
Protein, % (14% m.b.)	13.4	13.5	12.6	12.3	12.0
Sedimentation value	58.5	69.0	44.5	42.5	30.0
Amylograph viscosity, B.U.	500	50	50	50	50
Starch damage (Farrand units)					
Farrand[a]	22.5	0.0	14.4	34.8	44.4
Williams and Fegol[b]	25.7	5.9	20.3	30.3	59.7
Farinogram					
absorption, %	67.1	58.8	58.9	56.4	54.3
development time, min.	3.5	3.0	2.0	1.5	1.0
M.T.I., B.U.	25.0	65.0	120.0	260.0	350.0
Baking absorption, %	63.1	54.8	51.9	...	...
Loaf volume, cc.	925	805	660	...	...
Loaf volume, cc. (0 malt)	800	870	610	...	...

[a]Cereal Chem. 41: 986 (1964).

[b]Cereal Chem. 47: 56 (1969).

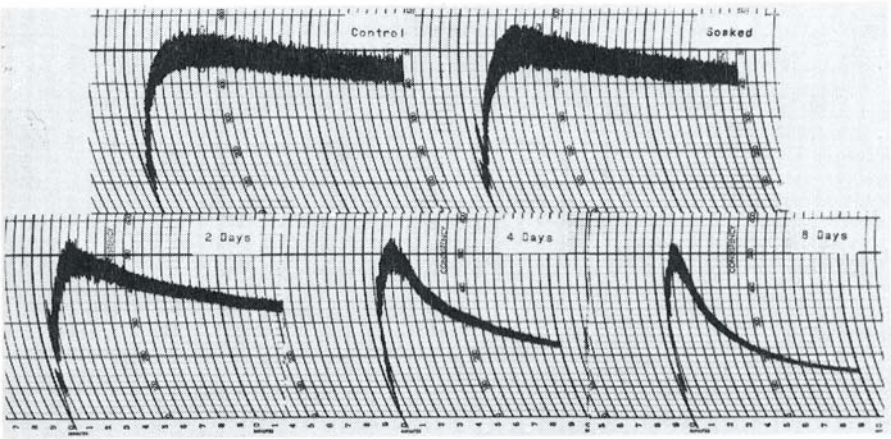

Fig. 1. Farinograms of flours milled from control, soaked, 2-, 4-, and 8-day germinated wheat.

amino acid analyzer. The hydrolysate was prepared in the usual manner using 6N hydrochloric acid and an 18-hr. hydrolysis under vacuum. Cysteine and cystine values were determined from sulfhydryl and disulfide contents. Amino groups were determined colorimetrically after reaction with ninhydrin reagent on the protein that was extractable from the flours with 1% acetic acid. Sulfhydryl contents of flours were determined by amperometric titration with silver nitrate (14). Disulfide contents were also determined by amperometric titration after reduction with bisulfite as described by Tsen and Anderson (15).

RESULTS AND DISCUSSION

To confirm the deterioration of breadmaking quality of flours from sprouted wheat, all five flour samples used in this study were subjected to normal technological tests (Table I). The amylograph viscosity of all flours, except the control, was about 50 B.U. These very low values are due to the high α-amylase activities of these samples (8). The zero-starch damage value of the soaked sample is interesting, but such low values are possible. The rapid deterioration of baking quality with sprouting is also indicated by the properties measured on the farinograph (Fig. 1). Farinograph absorptions for control, soaked, and the three sprouted samples were 67.1, 58.8, 58.9, 56.4, and 54.3%, respectively. Dough development time decreased markedly with sprouting. Using the remix baking test, only three of the flours (control, soaked, and 2-days) could be baked into bread, and only the first two gave satisfactory loaves. The 4- and 8-day samples gave doughs that were too sticky to handle.

Solubility Fractionation of the Flour Proteins

The distribution of protein (Kjeldahl nitrogen × 5.7) among the five solubility fractions is given in Table II. A considerable increase in the proportion of water-soluble protein was obtained when the wheat was soaked prior to sprouting. This specific increase appears to be due to solubilization of proteins normally

TABLE II. SOLUBILITY FRACTIONATION OF FLOUR PROTEINS

Protein Fractions	Control %	Soaked %	Germinated 2 Days	Germinated 4 Days	Germinated 8 Days	S.D.[a]
Water-soluble	7.9	11.9	6.5	6.7	5.1	0.5
Salt-soluble	4.1	3.0	1.5	2.5	1.2	0.3
Ethanol-soluble	43.7	38.3	37.4	42.2	38.7	3.6
Acetic acid-soluble	11.6	12.6	18.3	24.2	28.5	2.8
Residue	26.7	29.0	25.3	11.1	7.2	1.0
Acetic acid-soluble + residue	38.3	41.6	43.6	35.3	35.7	...
Recovery	94.0	94.8	89.0	86.7	80.7	...

[a]Standard deviation based on five fractionations.

extracted in the 70% ethanol solution (gliadins). There was a slight decrease in this fraction with sprouting compared with that of the control. The amount of the salt-soluble fraction decreased during soaking and sprouting. On the other hand, the amount of the alcohol-soluble proteins remained rather constant (within experimental error) for all five flours. The most notable change observed in the solubility pattern was the marked increase in the amount of the acetic acid-soluble fraction and a parallel decrease in the residue proteins. The sum of the latter two fractions showed only a small decrease with sprouting. There was a gradual decrease in the total protein recovery in the five fractions with increased sprouting. This is attributed to the loss of low-molecular-weight nitrogen compounds in the dialysis step used to separate the water-soluble from the salt-soluble proteins. These results show that the major change occurring in the endosperm proteins during sprouting is the gradual degradation of the residue protein to give low-molecular-weight peptides (lost during dialysis) and acetic acid-soluble proteins. This is extremely important technologically since it was shown recently that loaf volume is directly related to the amount of residue protein and indirectly to the amount of acetic acid-soluble protein (16).

Chromatographic Fractionation on Sephadex G-150

Total AUC extracts were fractionated chromatographically on Sephadex G-150. The amount of protein solubilized by AUC was approximately 98% for the five flours used in this study. Elution profiles for these extracts are shown in Fig. 2. As the degree of sprouting increased, the proportion of fraction-I proteins decreased while that of group IV increased. The changes in the two intermediate fractions were relatively smaller and showed no definite trend with increasing sprouting. These results generally agree with the solubility fractionation data presented above.

Each solubility fraction from the five flours was dissolved in AUC and chromatographed. The areas of the same peak (designated by Roman numerals) for the five fractions were summed for each flour (Table III). The last column gives the sum of fraction IV and the amount of proteinaceous material in the dialysate determined by subtracting the total recovery (shown in parentheses for each flour) from 100.

As expected, data showed that the amount of the high-molecular-weight components (fractions I and II) gradually decreased with sprouting while the

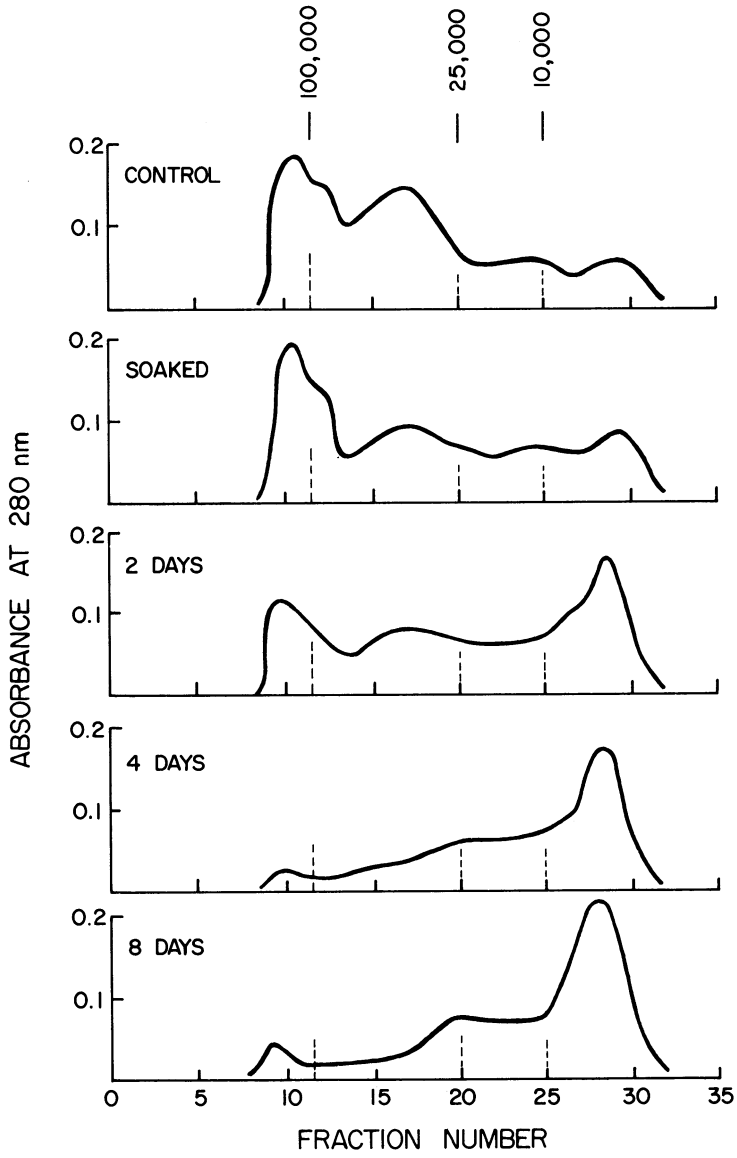

Fig. 2. Elution profiles of AUC extracts of the five flours.

amounts of the smaller components (fraction III and fraction IV plus dialysate) increased. The fraction-II proteins (gliadins) showed the greatest decrease. These results appear to contradict the data obtained by solubility fractionation (Table II), which showed that the alcohol-soluble fraction remained essentially constant while the residue fraction decreased markedly. However, fraction II appears to be quite different from the alcohol-soluble fraction (see PAGE results below). The results of

TABLE III. SUMMARY OF FRACTIONATIONS OF THE SOLUBILITY FRACTIONS ACCORDING TO MOLECULAR WEIGHT (M.W.)

	I M.W. > 100,000	II 100,000-25,000	III 25,000-10,000	IV < 10,000	IV + Dialysate
Flour					
Control (94.0[a])	29.9	55.7	5.6	2.8	8.8
Soaked (94.8)	35.1	50.5	7.7	2.5	7.7
Germinated					
2 days (89.0)	25.6	48.4	14.1	0.9	11.9
4 days (86.7)	27.1	46.3	12.4	0.9	14.2
8 days (80.7)	24.4	39.7	14.7	1.8	21.2

[a]Total protein recovery (% of flour protein) by the solubility fractionation.

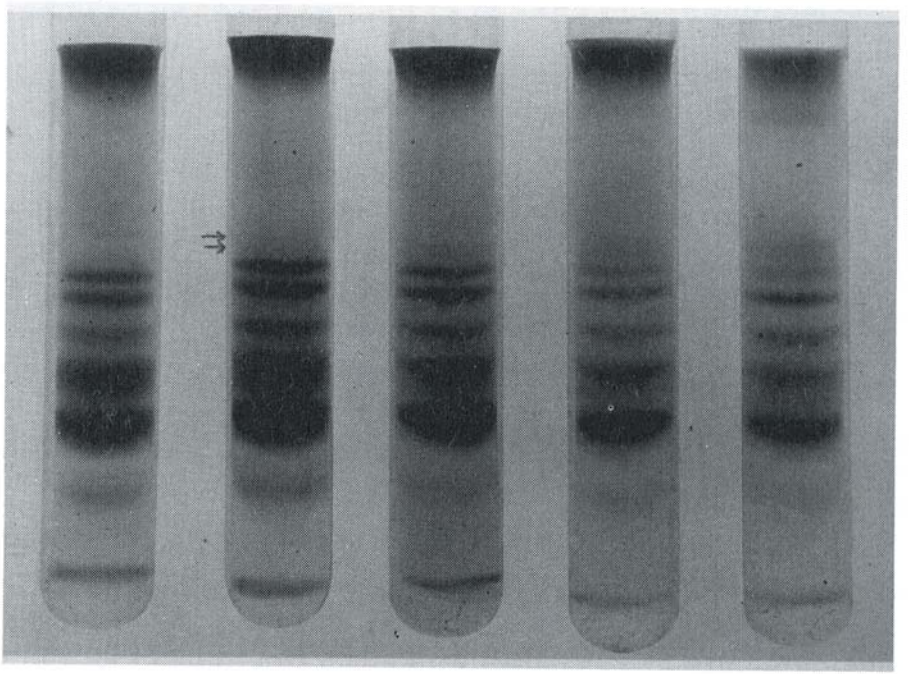

Fig. 3. Electrophoretic patterns of water-soluble proteins.

Table III calculated from chromatographic results of solubility fractions and the data obtained by gel filtration of total AUC extracts of flour (Fig. 2) both showed the same trends with sprouting. The small discrepancy between the two sets of data is attributed to the fact that different solvents were used for the initial extractions. In general, these results agree with those published by Coulson and Sim (17), Shorina and Vakar (18), and Beresh (4) who showed by different experimental techniques that gluten proteins are rapidly degraded during sprouting of wheat.

PAGE Results

These experiments were carried out to investigate the possibility of the formation of major "new" protein components during sprouting. Figure 3 shows the electrophorectic patterns for the water-soluble fractions of the five flours used in this study. The patterns for the soaked and sprouted samples showed two minor bands (indicated by arrows) in addition to the six bands of the control. These new bands could be the same as the additional bands observed in the fast-migrating components of Tris-HCl buffer extracts examined by Macko et al. (19). Coulson and Sim (17), who used starch gel electrophoresis to examine total acetic acid extracts, also observed a few "new" components of higher mobility formed during sprouting.

The electrophoretic patterns of the salt-soluble fraction are shown in Fig. 4. Bands of very slow mobility disappeared gradually during sprouting while all the fast-moving bands remained essentially unchanged. There was no change in the electrophoretic patterns of the alcohol-solubles (Fig. 5).

The four fractions obtained by chromatographic fractionation were also examined with the PAGE technique. Electrophoretic patterns for the control and 8-day sprouted samples are shown in Figs. 6 and 7, respectively. The patterns for the two samples are essentially the same, and therefore only results for the control will be discussed and minor differences noted. The two "new" bands in the water-soluble fraction noted above were not seen in the patterns of the chromatographic fractions.

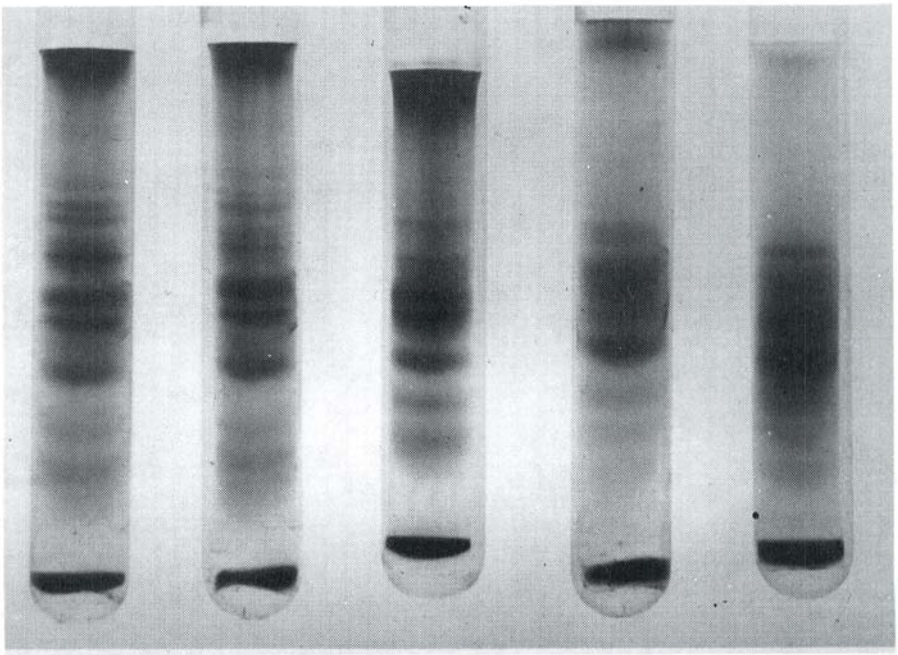

Fig. 4. Electrophoretic patterns of salt-soluble proteins.

Fraction I comprised mainly proteins that did not enter the polyacrylamide gel. This is generally true of glutenin proteins. This fraction contained a number of minor slow-moving or gliadin-like components. Fraction II comprised mainly slow-moving (gliadin) components and small amounts of protein that did not enter the gel and some fast-moving components (albumins or globulins). The gliadins by gel chromatography (fraction II) are not entirely equivalent to the gliadins obtained by solubility fractionation when the two are compared by PAGE. Fraction II is definitely not as homogeneous electrophoretically as the alcohol-soluble fraction (compare patterns for fraction II in Figs. 6 and 7 and Fig. 5).

Fraction III comprised entirely fast-moving bands with mobilities usually obtained for the water- and salt-soluble proteins. Fraction IV of the control flour contained one component of very high mobility. This component appears similar in mobility to one of the major components of fraction III. The amount of protein in fraction IV was very low. On the other hand, the fraction IV of the sprouted sample showed no protein bands. Presumably the small amount of material that absorbed at 280 nm. (Fig. 2) either moved very rapidly and off the gel, or it does not stain with amido black used to detect the protein bands. It can be concluded from the results presented in this section that, during sprouting, the endosperm proteins are either very quickly degraded to very low-molecular-weight peptides and amino acids or that the products of lower molecular weight degradation are electrophoretically identical to protein components normally present in flour.

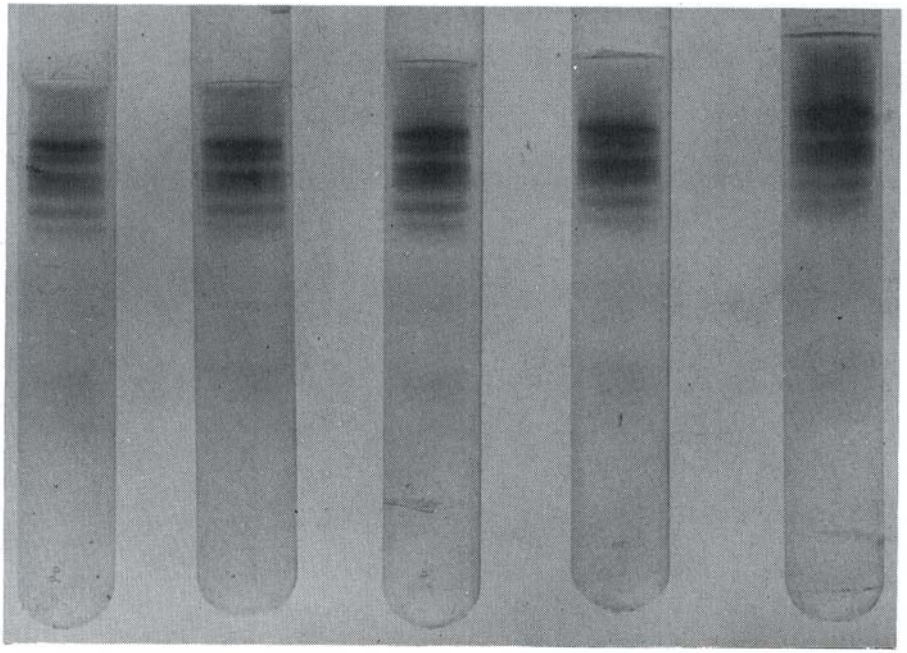

Fig. 5. Electrophoretic patterns of alcohol-soluble proteins.

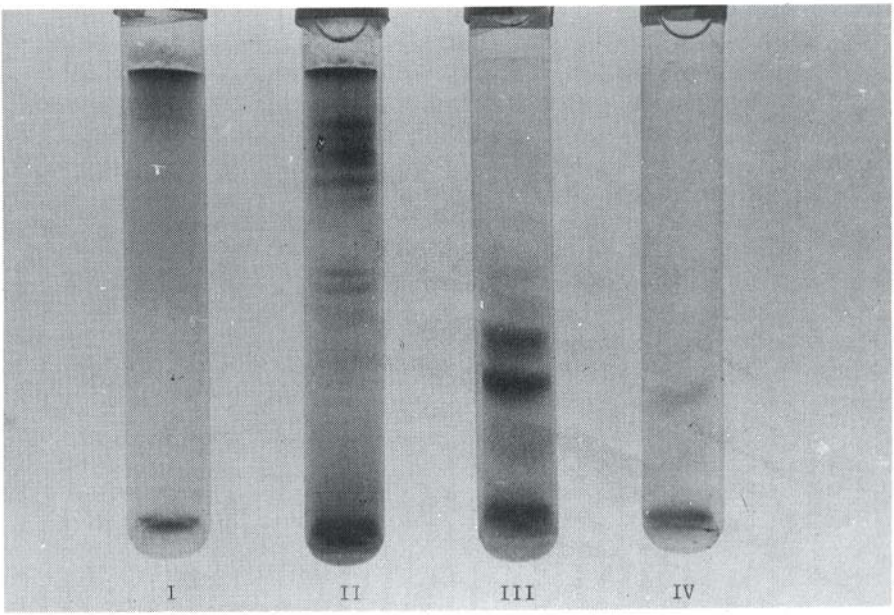

Fig. 6. Electrophoretic patterns of the four gel chromatographic fractions for the control sample.

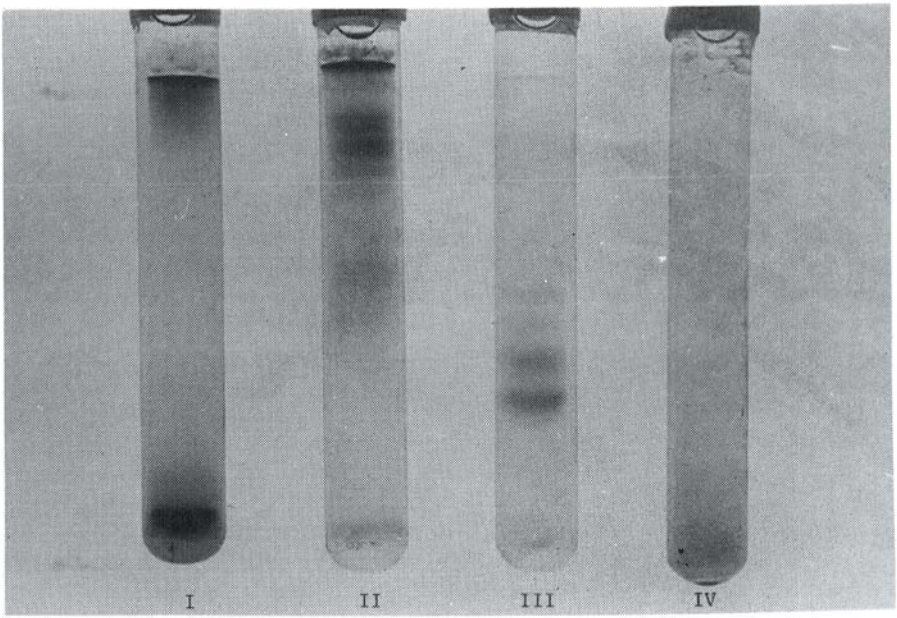

Fig. 7. Electrophoretic patterns of the four gel chromatographic fractions for the 8-day sample.

Proteolytic Activity

Activity of Flour. Activities of two different crude enzyme extracts from the five flours were determined. Both extracts showed a nonlinear increase in activity with duration of sprouting (Table IV). Two-day soaking had no effect on the proteolytic activity and 2 days of sprouting produced a relatively small increase. In contrast, these samples showed a marked increase in α-amylase over that of the control (8). Sprouting beyond 2 days produced a rapid increase in proteolytic activity. Activity of the extract from the 4-day sample was five to seven times the activity of the 2-day sample, and activity of the 8-day sample was more than twice the activity of the 4-day sample. The rate of increase in activity with sprouting was essentially the same for the two extracts. However, the activity of the acetate buffer extracts was approximately twice the activity of the ammonium sulfate extracts. Similar increases in proteolytic activity during sprouting were published by Mounfield (2).

Activity of Solubility Fractions. The water- and salt-soluble fractions had the highest specific activities (Table V). However, on the basis of total activity, the other two fractions (acetic acid-soluble and -insoluble) still retained considerable activity. Specific activities of the first two fractions increased during sprouting. The increase in total activity of the water-soluble fraction was exceptionally high, arising from the increases in specific activity and in the amount of protein in this fraction. Although the increase in specific activity of the water- and salt-soluble fractions was somewhat lower than the increase obtained for the two flour extracts discussed above, the general agreement between the two sets of results is satisfactory considering the difference in the two enzyme preparations (flour extracts versus freeze-dried solubility fractions). The water-soluble fraction could possibly have lost a portion of its activity during the lengthy extraction and dialysis periods necessary in the solubility fractionation. The salt-soluble fraction contained considerable activity. However, its specific activity increased only about twofold after 8 days of sprouting. The proteinases that develop during sprouting apparently are mainly of the water-soluble type.

The alcohol-soluble fraction had essentially no proteolytic activity as determined by the assay used in the present study. This observation appears contrary to the results of Kaminski and Bushuk (20) who showed that this fraction contained proteolytic activity that could be readily detected by the highly sensitive starch gel electrophoretic technique. This discrepancy is attributed to the difference in the sensitivity of the two methods used for detecting proteolytic activity. Both the acetic acid-soluble and residue proteins contained small amounts of proteolytic activity, possibly because of contamination of these fractions with water-soluble proteins.

The sum of the activities of the solubility fractions was approximately 60% of the activity of the more active single (acetate buffer) extract of each flour. These results are not surprising, since a loss of activity during the multi-step solubility fractionation involving dialysis and freeze-drying is expected.

Analyses on Flours

Table VI lists the amino acids that showed significant changes during sprouting. Arginine decreased during sprouting. Cysteine also showed a small, but significant, decrease. Of the other amino acids, proline showed a gradual decrease during sprouting while cystine showed an increasing trend. The increase in cystine for a

TABLE IV. PROTEOLYTIC ACTIVITY OF EXTRACTS FROM FLOUR OBTAINED WITH TWO SOLVENTS

	Activity			
	10% Ammonium Sulfate		Acetate Buffer	
	A[a]	B[b]	A[a]	B[b]
Control	1.5	1.0	3.4	2.2
Soaked	1.4	0.9	3.5	2.2
Germinated				
2 days	1.6	1.1	4.6	3.2
4 days	11.0	7.8	21.6	15.3
8 days	25.0	17.9	52.5	37.8

[a]Activity in (μmoles tyrosine per g. flour per min.) X 10^3.

[b]Activity in (μmoles tyrosine per mg. protein per min.) X 10^5.

TABLE V. PROTEOLYTIC ACTIVITY OF FRACTIONS OBTAINED BY SOLUBILITY FRACTIONATION

	Water-Soluble		Salt-Soluble		Alcohol-Soluble		Acetic acid-Soluble		Residue	
	Specific activity[a]	Total activity[b]	Specific activity[a]	Total activity[b]	Specific activity[a]	Total activity[b]	Specific activity[b]	Total activity[a]	Specific activity[a]	Total activity[b]
Control	10.3	81.3	14.7	60.3	...	...	1.5	17.4	2.7	72.1
Soaked	10.1	120.2	14.7	44.1	...	...	2.0	25.2	1.3	37.7
Germinated										
2 days	12.7	82.5	12.4	18.6	...	...	2.1	38.4	1.1	27.8
4 days	29.6	198.3	17.7	44.2	trace	trace	2.9	70.2	1.1	12.2
8 days	115.0	586.5	26.5	31.8	trace	trace	2.8	79.8	0.9	6.5

[a](μmoles tyrosine per mg. protein per min.) X 10^5.

[b](μmoles tyrosine per min.) X 10^5.

TABLE VI. AMINO ACIDS AFFECTED BY SPROUTING

	Arginine	Cysteine[a]	Cystine[a]	Proline
	g. amino acid N per 100 g. sample N			
Control	6.92	0.06	1.23	10.10
Soaked	6.83	0.06	1.39	9.65
Germinated				
2 days	6.15	0.04	1.63	9.96
4 days	5.99	0.04	1.39	9.61
8 days	6.14	0.04	1.49	9.57

[a]Calculated from SH and S-S contents.

specific sample was not equivalent to the decrease in cysteine.

Flour from soaked wheat had a slightly higher SH content than the control (Table VII). Its content decreased by 20 to 30% during sprouting. There was essentially no difference in the values for the 2-, 4-, and 8-day samples. The disulfide contents of the flour from the soaked and sprouted samples were slightly higher than the control. Because of sampling errors, little significance can be attached to the absolute differences in SH and S-S contents of the five samples. These results differ from those obtained for washed-out gluten by Shorina et al. (7) who found a decrease in S-S content with sprouting. The discrepancy could arise from the difference in experimental material (washed-out gluten versus total flour proteins). The SH and S-S groups do not appear to be involved in the degradation of wheat endosperm proteins that occurs during sprouting.

Table VII also shows the change of amino groups during sprouting. The number of amino groups increased rapidly during sprouting, indicating a significant hydrolysis of peptide bonds. In general, these results agree with those of Beresh (4) who showed that flour from wheat sprouted for 4 days had about eight times as many free amino groups as the flour from dormant wheat.

GENERAL DISCUSSION

One variety of Canadian hard red spring wheat (Manitou) was germinated and sprouted under controlled laboratory conditions and then used to study the effects of sprouting on some technological and biochemical properties. The changes in starch and α-amylase activity in the same wheat samples were discussed in a companion article (8).

TABLE VII. SH, S-S, AND NH_2 CONTENTS

	SH μeq. per g. flour	S-S μeq. per g. flour	NH_2 μmoles per mg. N
Control	0.91	10.3	1.75
Soaked	0.98	11.8	2.55
Germinated			
2 days	0.67	12.9	4.18
4 days	0.60	10.7	7.17
8 days	0.62	11.2	10.18

Milling flour yield on the Buhler experimental mill and flour color decreased with increasing degree of sprouting. Ash content of the flours from the three sprouted wheats was actually lower than the ash content of the control. Presumably, this decrease resulted partly from leaching during soaking prior to sprouting and partly from utilization of inorganic ions in the metabolism of the young wheat plant.

As observed by others (1), baking quality deteriorated extensively during sprouting. It is well known that this damage, when not too extensive, can be corrected by adjusting processing parameters such as water absorption, fermentation time, and proofing time. However, even with these adjustments bread quality obtained from flour milled from sprouted wheat is usually only marginal. The poor baking quality of flour from sprout-damaged wheat has been generally attributed to the development of high α-amylase activity (1).

The most important component of flour relative to its baking quality is protein. The present study showed that protein undergoes marked changes during sprouting which could have a further (in addition to α-amylase) detrimental effect on baking quality. First of all, there was a small loss of protein during sprouting as indicated by the flour's protein content. Secondly, the solubility properties were markedly affected (Table I). The extensive decrease in the amount of the residue component is probably the most important change in relation to baking quality. Orth and Bushuk (16) have shown that baking quality of 26 normal wheats of different type, expressed on the basis of loaf volume, was directly related to the amount of residue protein. The amount of this fraction decreased markedly during sprouting.

The observed changes in the solubility properties can be explained in terms of hydrolysis of peptide bonds by the increased proteolytic activity that develops during sprouting (Table V). Hydrolysis of peptide bonds during sprouting was confirmed by two sets of experimental evidence (Table III). Firstly, gel filtration chromatography showed a gradual decrease in the amount of the high-molecular-weight fraction (I) and a concomitant increase in the two low-molecular-weight fractions (III and IV). Secondly, the number of free amino groups increased rapidly with sprouting. For each sample, both decrease in molecular weight of proteins (decrease in the amount of residue and fraction I) and increase in amino nitrogen were related to proteolytic activity. It is technologically significant that the high-molecular-weight component of the endosperm proteins was degraded preferentially to the other components. The heavy component is essential for formation of gluten that will have optimum gas-retaining capacity in bread doughs.

Other minor analytical changes in the proteins during sprouting were observed. Amino acid compositions showed significant decreases in the proportions of arginine and proline. Analyses of sulfhydryl (SH) and disulfide (S-S) groups showed a slight decrease in SH and a small increase in S-S, which could be technologically significant because of their critical functional role in dough.

The present study confirms and extends recently published work from the U.S.S.R. (4) which showed that degradation of gluten proteins during sprouting occurs as a result of cleavage of peptide bonds. No evidence was obtained in the present study to support the hypothesis of Shorina et al. (7) that the breakdown of gluten occurs primarily through the reduction of S-S bonds by protein disulfide reductase. Accordingly, it is concluded that the breakdown of the gluten complex during sprouting starts with cleavage of peptide bonds. Subsequently, this could

lead to a breakdown of secondary (ionic, hydrogen, hydrophobic) bonds known to contribute to the physical structure of gluten. In relation to breadmaking technology, some proteolytic hydrolysis occurs in the wheat kernel and subsequently additional breakdown occurs during the breadmaking process when proteolytically active flour is mixed into a dough.

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